

RE-EVALUATION OF TAXONOMIC CHARACTERS OF *IDIOSEPIUS* (CEPHALOPODA, MOLLUSCA)

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ABSTRACT

Members of the cephalopod genus *Idiosepius* Steenstrup, 1881, are currently mainly identified based on the arrangement of suckers on the tentacular club (i.e., in two or four transverse rows) and the number of suckers on the male hectocotylized ventral arms, as stated by Nesis (1982). However, the discovery of a further species, *I. thailandicus*, raises questions about the validity of the present systematic characters. Alternative morphological features, such as the mantle length and peg arrangement on the tentacle suckers, were proposed by Chotiyaputta et al. (1991) to be suitable for species classification.

To determine whether these attributes are more reliable diagnostic characters valid for all *Idiosepius* species, we re-evaluate all the above taxonomic characters as well as others features, such as the number of suckers on the other extremities, the shape of the hectocotyli, radula morphology, and beak size.

Based on the present analysis, the systematic positions of *I. macrocheir* and *I. thailandicus* are uncertain, although they both correspond morphologically to *I. biserialis*.

The current evaluation supports a revision of the systematic key of Nesis (1982). The data presented here indicate that the species should be characterized by the shape of the hectocotylus and its appendages rather their sucker number. All other evaluated morphological attributes – sucker and its peg arrangement, mantle size, beak and number of suckers in the first three arm pairs – do not provide good characters to discriminate the species of *Idiosepius*.

Key words: classification, hectocotylus, Idiosepiidae, morphology, revision, species characterization, systematic key.

INTRODUCTION

Members of the genus *Idiosepius* Steenstrup, 1881, are the smallest of all cephalopods, with adult mantle lengths of less than 3 cm in females and 1 cm in males (Hylleberg & Nateewathana, 1991 a). One conspicuous morphological character in representatives of this monogeneric family (Idiosepiidae Appellöf, 1898) is the adhesive organ (also known as adhesive gland) located on the posterior portion of the dorsal mantle (Sasaki, 1921; Steenstrup, 1881). Studies on this genus deal with this cephalopod's behavior and development, as well as the morphology of the adhesive organ (Nabhitabhata, 1998; Shigeno & Yamamoto, 2002; Byern et al., 2008). The relationships among species within this family, however, are still unknown.

Jereb & Roper (2005) currently place eight species within the genus *Idiosepius*: *Idiosepius*

biserialis Voss, 1962; *I. macrocheir* Voss, 1962; *I. minimus* (d'Orbigny, 1845); *I. notoides* Berry, 1921b; *I. paradoxus* (Ortmann, 1888); *I. picteti* (Joubin, 1894); *I. pygmaeus* Steenstrup, 1881; and *I. thailandicus* Chotiyaputta et al., 1991. These species are mostly distributed in the tropical Indo-Pacific, Japan, southern Australia, including Tasmania and African waters, but one was found in cooler Russian and Japanese waters (Nesis et al., 2002; Sato et al., 2009). Within the genus, *I. biserialis* has the widest geographical distribution, ranging from Japan to the Indo-Pacific, including Thailand and Indonesia (Byern et al., 2005; Voss, 1963); it has also been recorded in Mozambique (incorrectly annotated by Voss in 1962 as "South Africa").

Nesis (1982) characterized the species of this genus by the arrangement of suckers on their tentacular club (*I. biserialis* with two rows, *I. notoides*, *I. macrocheir*, *I. paradoxus*, *I. picteti*

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and *I. pygmaeus* with four rows) and the number of suckers on the male hectocotylus. However, in his systematic key, only six of the above-mentioned eight species are represented; *Idiosepius thailandicus* was described later by Chotiyaputta et al. (1991). For *I. minimus*, no type material or any detailed morphological descriptions are available to confirming its validity. Nevertheless, this species is mentioned in previous (Berry, 1921a, b) and recent discussions (Jereb & Roper, 2005) of this genus.

The discovery of *I. thailandicus* raises questions about the validity of the above-mentioned systematic characters; this species closely resembles *I. biserialis*, likewise collected by Hylleberg & Nateewathana (1991a) in Thai waters, in having a biserial arrangement of suckers on the tentacle clubs and similar number of suckers on the ventral arms. Chotiyaputta et al. (1991) proposed to differentiate these species by mantle length (*I. thailandicus* slightly smaller than *I. biserialis*) and sucker ultrastructure. Whereas *I. thailandicus* tentacle suckers have two series of pegs that become triple at the distal portion of the club, *I. biserialis* shows a distinct threefold circular arrangement of pegs throughout on the suckers.

To date, however, it remains unverified whether these morphological features (mantle length and sucker ultrastructure) are reliable characters for species other than *I. biserialis* and *I. thailandicus*.

To address this problem, we re-evaluate all taxonomic characters proposed by Nesis (1982) and Chotiyaputta et al. (1991) in all nominal *Idiosepius* species and compare these features against each other. Additionally, we evaluate the suitability of the number of suckers on the other extremities as well the shape of the hectocotyli, radula morphology and beak size.

The purpose of this study is to define easily detectable but reliable morphological characters suitable to classify all nominal species (except *I. minimus*) of the genus *Idiosepius*, and here we aim to contribute to the taxonomic understanding of the pygmy squids, but we also rely on the support of colleagues working on this genus to help us re-organize the systematic key of *Idiosepius*.

MATERIAL AND METHODS

Some of the data presented here are already partially mentioned in the original literature. Those original data are mainly based on a small sample size from one locality.

Samples

All samples presented here were collected by the first author with the help of local partners and with official national collection permissions. In most cases, numerous individuals of species obtained from different localities were likewise evaluated. The number of individuals of each taxon is listed in Tables 1–6.

In order to provide a more complete survey, data of Chotiyaputta et al. (1991), Joubin (1894), Tracey et al. (2003) and Voss (1962) were included when necessary.

Type Material

Re-examination of all type material, apart from *I. macrocheir* (SAM A6521) and *I. picteti* (M 3/75 747/27), was not possible because of the risk of damaging the unique material. Attempts to re-collect these species in their original type locations were unsuccessful: only specimens of *I. pygmaeus hebereri*, collected by Grimpe (1931), could be collected at the original locality by Byern & Klepal (2007). The collected specimens were examined and identified according to the systematic key of Nesis (1982) as well as the original reference literature.

Morphological Analysis

Live specimens were kept in seawater tanks and fed ad libitum with mysids and shrimps. For all collected specimens, mating behavior and egg spawning in aquaculture were observed during sample collection and cultivation, indicating that all animals included in this study were mature. For the subsequent analyses, the animals were anesthetized with 3% ethanol-seawater (v/v) solution, measured, and decapitated.

The body measurements include mantle length, mantle width, and mantle width index, according to Roper & Voss (1983). Measurement of the arm and tentacle length was inappropriate because the appendages were retracted. The mantle with its adhesive organ was isolated and fixed for other investigations (Byern et al., 2008); the head was fixed in 70% EtOH. Some specimens of *I. biserialis* from Mozambique and *I. pygmaeus* from Indonesia were not anesthetized or measured, but were immersed directly in 70% EtOH.

Of each collection place, some individuals of each species were kept intact and given to the collection of the Natural History Museum Vienna (Table 1), but not evaluated in the

TABLE 1. Specimen and collection information for *Idiosepius* spp. (*Museum abbreviations according to Integrated Taxonomic Information System-ITIS; # in the collection of the Muséum d'Histoire Naturelle, Genève, Switzerland, instead of the Musée Océanographique, Monaco; + re-numbered and stored in the PMBC since 2009).

Species	Location	Coll. Date	Reference	Mus. Ref. No.*
<i>I. biserialis</i>	San Jose Mission Station, Morrumbene, Mozambique (Holotype)	June 1954	Voss, 1962	SAM A6520
	Bang Rong, Phuket Island, Thailand (8°02.156'N; 98°25.487'E)	April 2004		NMW 103228-103229
	Inhaca Island, Mozambique (26°00.215'S; 32°54.721'E)			
	Inhambane Bay, Mozambique (23°51.184'S; 35°22.553'E)	October 2004	Byern et al., 2008	NMW 103265-103268
	Ekas-Bay, Lombok, Indonesia (08°52.020'S; 116°27.541'E)	May 2004		
	Takasu, Japan	August 2000	Byern et al., 2005	-
<i>I. macrocheir</i>	Linga Linga, Morrumbene, Mozambique (Holotype)	June 1954	Voss, 1962	SAM A6521
	Vilanculos, Mozambique	May 1973		SAM 186
<i>I. notoides</i>	Goolwa, South Australia (Holotype)		Berry, 1921a	SAMA D17495
	Fortescue Bay, Tasmania Australia (43°08'S; 147°57'E)	May-June 2000	Tracey et al., 2003	-
	Margate, Tasmania, Australia (43°02.969'S; 147°16.034'E)			-
	Snug, Tasmania, Australia (43°04.357'S; 147°15.943'E)	February 2006	Byern (unpubl. data)	-
<i>I. paradoxus</i>	Kadsiyama, Bay of Tokyo, Japan (Holotype)		Ortmann, 1888	MZS Mol 5809
	Nagoya, Japan (34°43.021'N; 136°58.208'E)			NMW 103227, 103273,
	Ushimado, Japan (34°35.567'N; 134°07.340'E)	April 2005	Byern et al., 2008	103274
<i>I. picteti</i>	Amboine Island, Indonesia (Holotype)		Joubin, 1894	MHNG M 3/75 747/27#
<i>I. pygmaeus</i>	Zamboanga, Philippines (4°20'N; 107°20'E) (Syntypes)		Steenstrup, 1881	ZMUC CEP-52
	Mudong, Phuket Island, Thailand (7°48.107'N; 98°24.472'E)		Byern et al., 2008	NMW 103230
	Bang Rong, Phuket Island, Thailand (8°02.945'N; 98°25.030'E)	April 2004	Suwanmala et al., 2006	NMW 103231
	Ekas-Bay, Lombok Island, Indonesia (08°52.020'S; 116°27.541'E)	May 2004	Byern et al., 2007	NMW 103223-103225
<i>I. pyg. hebereri</i>	Lombok Island, Indonesia (Holotype)	April 1927	Grimpe, 1931	ZMB Moll. 86118a
<i>I. thailandicus</i>	Donsak Surat-tani, Thailand (Lectotype)			PMBC 25267+
	Donsak Surat-tani, Thailand (Paratype)	May 1989	Chotiyaputta et al., 1991	NSMT Mo 69624 No. 4

present analyses. Most samples are still in the private collection of the first author.

All suckers on the arms and tentacles of the EtOH-fixed specimens were counted using a stereomicroscope (limited to 10 individuals of each sex, species and location). If sucker cups were missing, then sucker pedicels were used to determine their position and these were included in the sucker counts.

The peg arrangement on the suckers – five individuals of each sex/species and from different locations – was examined on different parts (basal, middle and near the tip) of all arms and tentacle clubs. A full statistical evaluation of the pegs of all suckers on arms and tentacles could not be undertaken, the unfavorable position of the differently orientated and the often sediment-occluded suckers preventing complete examinations. Moreover, the length of arms or tentacles could not be measured because of their contraction due to anesthesia and fixation.

For scanning electron microscopic (SEM) analyses, arms, and tentacles were dehydrated in a graded series of ethanol, washed several times in acetone, immersed in HMDS (hexamethyldisilazane), dried overnight in air, mounted on stubs, coated with gold in a Polaron 5800 sputter coater, and examined using a Philips XL 20 SEM.

Beaks were isolated by enzymatic digestion with collagenase IV (Cat #17104-019, Invitrogen, Carlsbad, California, USA) (100 units/ml with phosphate buffer solution, pH 7.4, plus 10% sucrose, shaken for 3–5 days at 37°C). Remaining tissue was removed manually; complete beaks were washed with Aqua bidest and immersed briefly in 1% osmium tetroxide to harden the chitin. Beak dimensions were measured using a dissecting microscope to 0.1 mm accuracy using the software program Lucia 5.10 (Co. Laboratory Imaging, Czech Republic). Measurements taken in all species include upper and lower hood length (UHL, LHL), upper and lower rostral tip to wing base (URW, LRW), upper and lower crest length (UCL, LCL) and lower baseline length (LBL), as defined by Clarke (1962, 1986).

Radulae were isolated by trypsin digestion (0.3% trypsin with 0.15% sodium sulphide for 24 h at 37°C), washed in Aqua bidest, immersed for 24 h in 1% osmium tetroxide, washed again, immersed in HMDS, and further treated for SEM analysis in the same manner as described above for the arms and tentacles.

Characterization of the adhesive organ (Cyran et al., 2005) and of its glandular composition (Byern et al., 2008) as well as reports on the

internal morphology in *I. biserialis* and *I. pygmaeus* (Hylleberg & Nateewathana, 1991a, b) provide no species-related differences.

Statistical representation of results includes means and standard error of means. The 95% confidence intervals of the means were calculated by adding to or subtracting from the mean the standard error of the mean times the theoretically expected t-value ($df = n-1$).

Statistical analysis of ANOVA and Mann Whitney U test was performed with the computer program SPSS (Version 18).

RESULTS

External Morphology

Apart from the commonly known body deformations due to the fixation process, anesthesia also influences the sucker arrangement on the tentacles (but not the arms) of *I. biserialis*, but not that of four-row species. In the anaesthetized state, the tentacle club of *I. biserialis* exhibits the so-far-described two-row arrangement (Fig. 1A), whereas the clubs in non-anaesthetized specimens widen and display a three to four-row sucker arrangement (Fig. 1B, C).

The sucker arrangement of the tentacle club of the female paratype of *I. macrocheir* (Fig. 1D) from the South African Museum (SAM 186) appears to be similarly widened and displays a three-row arrangement.

Mantle Length

Culturing animals for a short time (up to 7–10 days) did not influence the size of *I. biserialis*, *I. paradoxus* or *I. pygmaeus*; the animals kept their size during this cultivation period. Figure 2 and Table 2 provide a detailed overview of size values in all *Idiosepius* species.

Within *Idiosepius biserialis* specimens from Mozambique and Thailand are intermediate in size, in contrast to the larger individuals from Japan and the smaller specimens from Indonesia.

Recent measurements of the holotype of *Idiosepius macrocheir* (SAM A6521) differ slightly from the data of Voss (1962) (original data given in brackets).

Idiosepius notoides from different locations shows size differences. Specimens from Coles Bay, collected by Tracey et al. (2003), are slightly larger than those from Fortescue Bay but bigger than individuals collected by the first author from Snug/Margate.

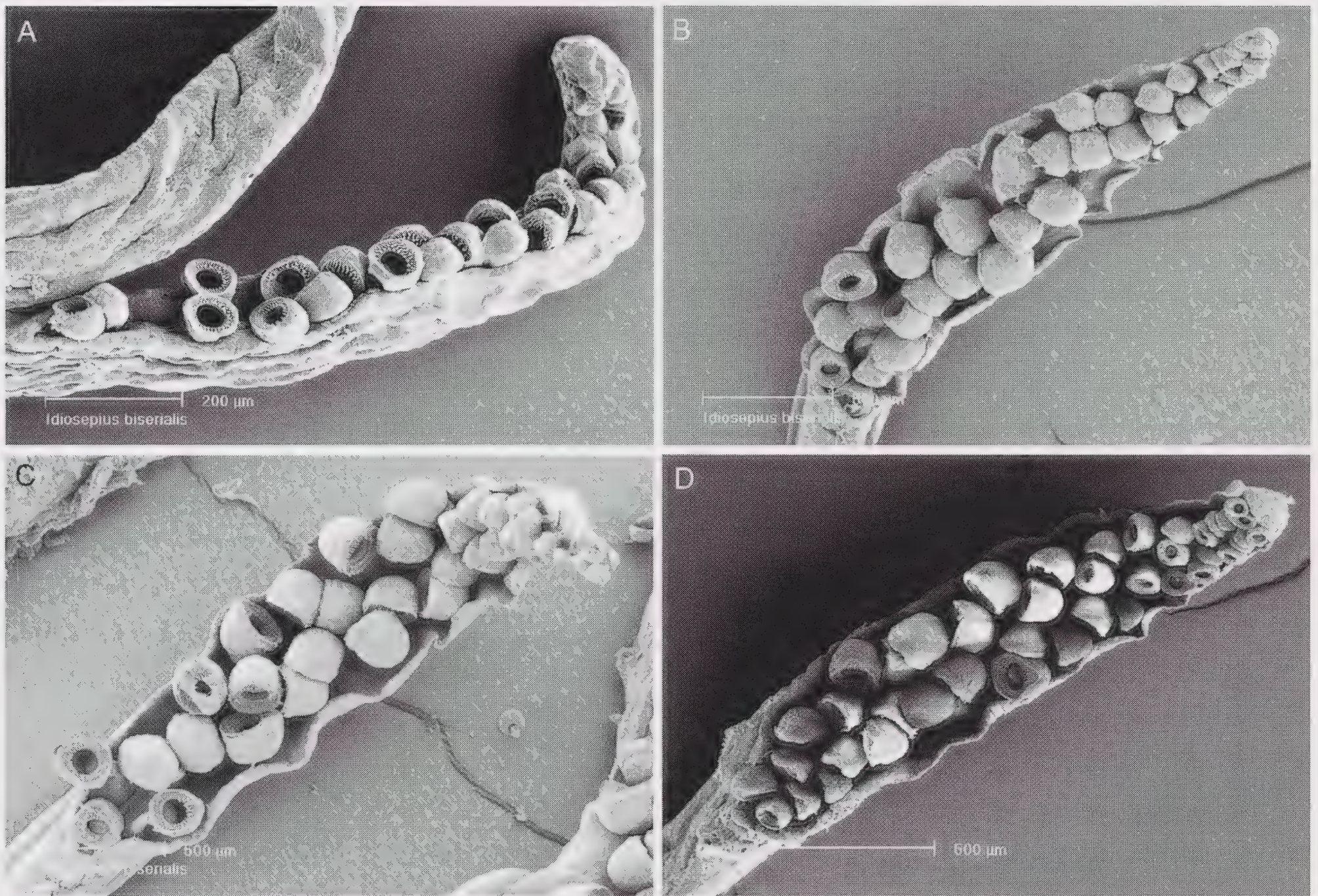


FIG. 1. Suckers of specimens of *Idiosepius biserialis*. (A): 2-rowed arrangement of suckers on the tentacle club; (B, C): 3- up to 4-rowed sucker arrangement; (D): Tentacle club of a female paratype (SAM No. 186) of *I. macrocheir* from the South African Museum.

Individuals of *Idiosepius paradoxus* from Ushimado are smaller than specimens from Nagoya.

The single holotype individual of *Idiosepius picteti* represents the largest male individual within the genus (Joubin, 1894).

The males of *Idiosepius pygmaeus* from Bangrong are almost the same size as those from Mudong. In contrast, a great size difference is apparent in the females from the two locations. Specimens from Bangrong are smaller than those from Mudong. Specimens from Indonesia are similar in size to individuals from Thailand.

Based on the data by Chotiyaputta et al. (1991), the individuals of *Idiosepius thailandicus* are larger than *I. biserialis* from Thailand.

Based on the present mantle length data, females are, regardless their geographical location and species, significantly larger than males, as pointed out in Table 3.

In summary, the results show that both sexes of the two-row species *I. biserialis* (♂ 5.60 mm $\pm$ 0.58 mm; ♀ 7.46 mm $\pm$ 1.04 mm) are

smaller than those of the four-row species *I. notoides*, *I. paradoxus*, *I. pygmaeus* (♂ 11.18 mm $\pm$ 1.53 mm; ♀ 14.32 mm $\pm$ 2.50 mm), whereas females are always almost 1/4–1/3 larger than males.

According to the mantle length, the single female of *I. macrocheir* (10.2 mm) lies between both groups, while *I. picteti* (14 mm) is clearly related to the four-row group.

Suckers on Extremities

Within an individual, each pair of arms and tentacles has a similar number of suckers (Table 4). On average, arm pair II has more suckers than arm pairs III or I. In males, arm pair IV is markedly hectocotylized and has fewer suckers, whereas in females the number of suckers on this arm pair is similar to the other arm pairs. On average, females have more suckers on the tentacle clubs than males. In the following, the total number of suckers on both tentacle clubs of all individuals within each species is used for comparison (Fig. 3).

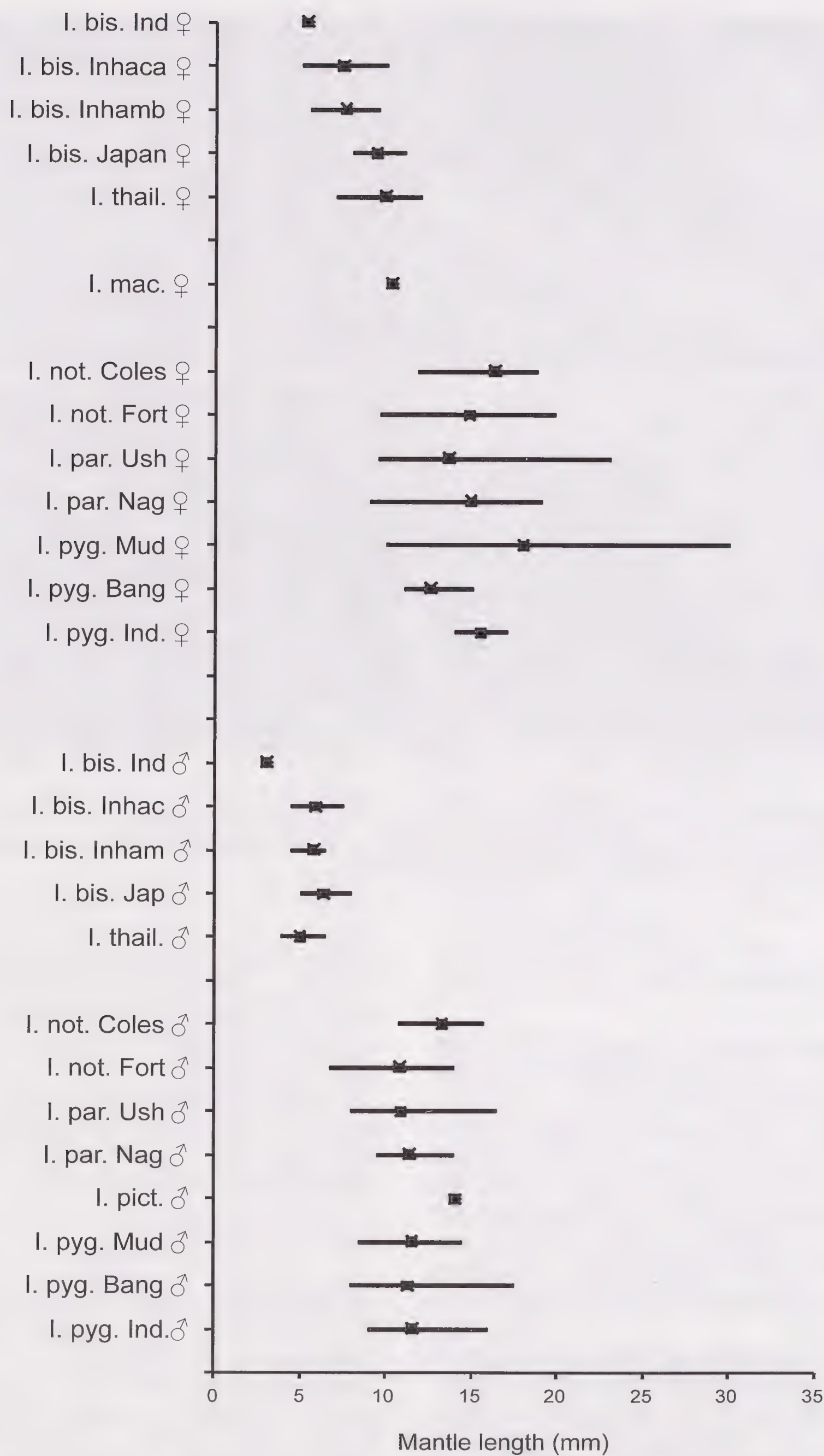


FIG. 2. Mean (■) and 95% confidence interval (n-1) (—) for the mantle length in all *Idiosepius* spp. For some species only a single specimen was available (*I. biserialis* Ind. ♂, *I. macrocheir* ♀ and *I. picteti* ♀), while in other specimens (*I. bis.* Indonesia ♀, *I.pyg.* Indonesia ♀, *I.pyg.* Bangrong ♀) the sample range was low (see Table 2).

TABLE 2. Size measurements in *Idiosepius* spp. specimens studied (■Data by Voss, 1962; +Data by Tracey et al., 2003; #Data by Joubin, 1894; *Data by Chotiyaputta et al., 1991).

Species	Mantle length (mm)		Mantle width (mm)		Mantle width index	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
<i>I. biserialis</i> ♀ Inhaca, Moçambique (n = 39)	7.38	1.31	4.09	0.63	56.16	8.11
<i>I. biserialis</i> ♂ Inhaca, Moçambique (n = 65)	5.84	0.54	3.24	0.34	55.74	6.12
<i>I. biserialis</i> ♀ Inhambane, Moçambique (n = 10)	7.60	1.33	4.15	1.36	54.63	15.74
<i>I. biserialis</i> ♂ Inhambane, Moçambique (n = 24)	5.71	0.57	3.02	0.10	53.37	5.02
<i>I. biserialis</i> ♀ Phuket Island, Thailand (n = 10)	7.70	1.10	4.20	0.80	55.10	3.50
<i>I. biserialis</i> ♂ Phuket Island, Thailand (n = 7)	4.50	0.30	2.20	0.20	48.90	4.60
<i>I. biserialis</i> ♀ Takasu, Japan (n = 4)	9.39	1.10	4.24	0.39	45.29	3.42
<i>I. biserialis</i> ♂ Takasu, Japan (n = 7)	6.34	0.89	3.06	0.50	48.46	6.27
<i>I. biserialis</i> ♀ Lombok, Indonesia (n = 2)	5.25	0.35	4.00	1.41	75.45	21.86
<i>I. biserialis</i> ♂ Lombok, Indonesia (n = 1)	3.00	-	2.50	-	83.33	-
Mean (♀)	7.46	1.04				
Mean (♂)	5.60	0.58				
<i>I. macrocheir</i> ♀ (n = 1) ■	10.20 (10.50)	-	5.32 (6.00)	-	52.16 (57.14)	-
<i>I. notoides</i> ♀ Coles Bay (n = 19)+	16.23	1.69	-	-	-	-
<i>I. notoides</i> ♂ Coles Bay (n = 38)+	13.30	1.20	-	-	-	-
<i>I. notoides</i> ♀ Fortescue Bay (n = 10)+	14.82	3.15	-	-	-	-
<i>I. notoides</i> ♂ Fortescue Bay (n = 14)+	10.78	2.13	-	-	-	-
<i>I. notoides</i> ♀ Snug/Margate (n = 8)	9.06	1.82	6.19	0.75	69.27	6.59
<i>I. notoides</i> ♂ Snug/Margate (n = 18)	8.67	1.90	5.86	0.74	69.57	11.06
Mean (♀)	13.37	2.22				
Mean (♂)	10.92	1.74				
<i>I. paradoxus</i> ♀ Ushimado, Japan (n = 27)	13.54	2.60	7.06	1.07	53.05	8.76
<i>I. paradoxus</i> ♂ Ushimado, Japan (n = 83)	10.93	1.32	5.4	0.66	49.78	6.36
<i>I. paradoxus</i> ♀ Nagoya, Japan (n = 23)	14.93	2.42	7.63	0.90	52.97	14.57
<i>I. paradoxus</i> ♂ Nagoya, Japan (n = 34)	11.37	1.01	5.53	0.63	48.88	6.27
Mean (♀)	14.24	2.51				
Mean (♂)	11.15	1.17				
<i>I. picteti</i> ♂ (n = 1)#	14.00	-	5.50	-	39.29	-
<i>I. pygmaeus</i> ♀ Mudong (n = 15)	17.97	4.04	8.53	1.14	48.40	6.33
<i>I. pygmaeus</i> ♂ Mudong (n = 89)	11.58	1.19	5.01	0.65	43.40	5.00
<i>I. pygmaeus</i> ♀ Bangrong (n = 3)	12.50	2.18	6.33	1.04	51.74	12.70
<i>I. pygmaeus</i> ♂ Bangrong (n = 25)	11.28	2.10	4.82	1.06	42.81	6.14
<i>I. pygmaeus</i> ♀ Lombok (n = 2)	15.50	2.12	7.50	2.12	47.90	7.13
<i>I. pygmaeus</i> ♂ Lombok (n = 42)	11.57	1.28	5.49	0.70	47.82	6.12
Mean (♀)	15.32	2.78				
Mean (♂)	11.46	1.55				
<i>I. thailandicus</i> ♀ (n = 13)*	9.85	-	5.72	-	58.10	-
<i>I. thailandicus</i> ♂ (n = 11)*	5.03	-	2.96	-	58.90	-

TABLE 3. Sexual dimorphism of mantle lengths in *Idiosepius* spp. Females are, regardless their geographical location and species, significantly larger than males.

Species	Model	df	F	Sig.
<i>I. biserialis</i> ♀ = 59 ♂ = 96	Corrected Model	1	131.737	0.000
	Intercept	1	7404.771	0.000
	Sex	1	131.737	0.000
<i>I. notoides</i> ♀ = 37 ♂ = 70	Corrected Model	1	20.374	0.000
	Intercept	1	1885.277	0.000
	Sex	1	20.374	0.000
<i>I. paradoxus</i> ♀ = 50 ♂ = 117	Corrected Model	1	110.088	0.000
	Intercept	1	7208.160	0.000
	Sex	1	110.088	0.000
<i>I. pygmaeus</i> ♀ = 18 ♂ = 56	Corrected Model	1	136.184	0.000
	Intercept	1	3642.924	0.000
	Sex	1	136.184	0.000

Idiosepius biserialis has 39.5 ± 5.09 (♀) and 32.11 ± 2.93 (♂) suckers on the clubs; males from Japan have more suckers on tentacles than animals from Thailand or Mozambique. In females, the ranking is the opposite: Mozambique more than Thailand and Japan.

The female holotype of *Idiosepius macrocheir* has 40 and 44 suckers on each tentacle club. *Idiosepius notoides* have the most suckers on the tentacle club within the genus (♀ 78.72 ± 4.33 and ♂ 62.82 ± 8.53). Notably, *I. notoides* has more injuries on the appendages than any

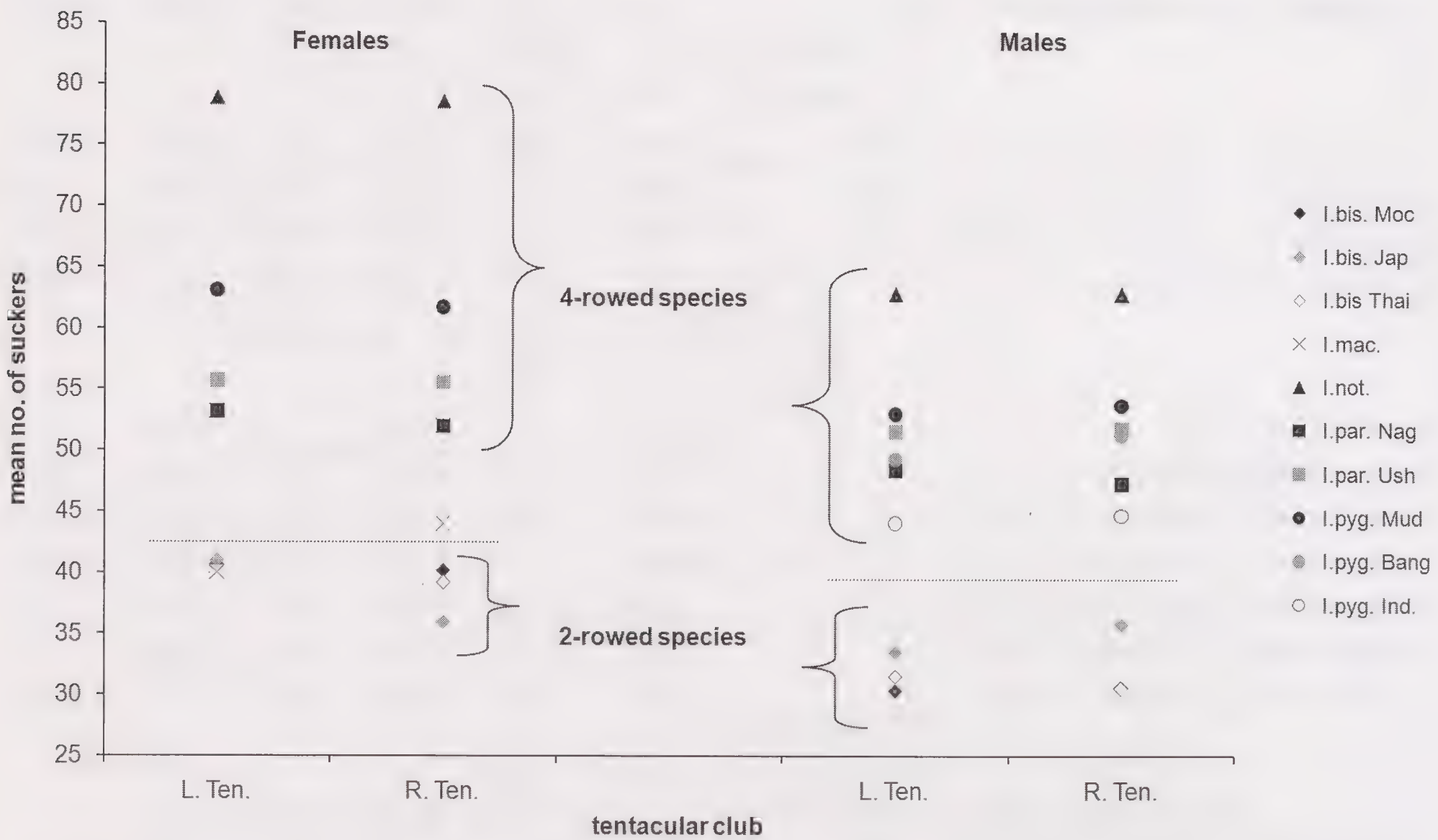


FIG. 3. Average number of suckers on the right (R. Ten.) and left tentacle club (L. Ten.) in females and males in *Idiosepius* spp.

TABLE 4. Number of suckers on appendages in *Idiosepius* spp. (*Data by Chotiyaputta et al., 1991).

Species		No. of suckers on appendages				
		A1	A2	A3	TC	A4
<i>I. biserialis</i> ♀ Moçambique n = 10	mean	23.95	26.75	25.95	40.40	26.70
	S.D.	3.02	1.91	2.42	3.61	3.52
	range	17-29	24-30	20-30	34-46	15-31
<i>I. biserialis</i> ♂ Moçambique n = 10	mean	17.95	21.45	20.30	30.55	4.30
	S.D.	1.61	2.34	2.63	3.44	0.84
	range	15-26	18-28	14-24	24-38	3-7
<i>I. biserialis</i> ♀ Thailand n = 10	mean	21.25	24.30	22.35	39.60	23.45
	S.D.	1.55		2.30	3.49	2.94
	range	19-24	21-28	16-26	34-46	17-28
<i>I. biserialis</i> ♂ Thailand n = 7	mean	14.64	17.72	16.57	31.07	3.79
	S.D.	1.92	0.90	1.27	1.86	0.94
	range	11-18	16-19	15-18	28-34	2-5
<i>I. biserialis</i> ♀ Japan n = 4	mean	16.88	18.50	18.75	38.50	20.00
	S.D.	3.71	2.95	2.99	8.16	1.79
	range	12-23	15-24	15-22	25-48	17-24
<i>I. biserialis</i> ♂ Japan n = 7	mean	17.65	20.07	18.43	34.72	4.15
	S.D.	1.42	2.27	2.02	3.51	0.54
	range	15-20	16-23	14-21	26-38	3-6
Mean (♀)		22.08 ± 2.50			39.50 ± 5.09	
Mean (♂)		18.31 ± 1.82			32.11 ± 2.93	
<i>I. macrocheir</i> ♂ n = 1	total	24+25	27+29	22+30	40+44	29
<i>I. notoides</i> ♀ Snug/Margate n = 8	mean	30.30	35.27	32.24	78.72	32.50
	S.D.	1.53	3.11	2.22	4.33	2.59
	range	27-35	32-40	27-36	70-83	29-38
<i>I. notoides</i> ♂ Snug/Margate n = 18	mean	26.25	30.97	27.75	62.82	17.31
	S.D.	2.82	2.62	3.20	8.53	2.44
	range	22-31	27-34	24-33	48-77	11-26
Mean (♀)		32.60 ± 2.29			78.72 ± 4.33	
Mean (♂)		28.32 ± 2.88			62.82 ± 8.53	
<i>I. paradoxus</i> ♀ Ushimado n = 10	mean	28.10	31.75	29.10	55.65	28.60
	S.D.	2.62	1.69	2.22	3.37	1.95
	range	21-33	28-34	24-32	51-62	25-31
<i>I. paradoxus</i> ♂ Ushimado n = 10	mean	25.50	28.35	26.25	51.70	6.45
	S.D.	2.84	2.73	2.54	6.02	1.06
	range	19-30	24-31	22-30	43-62	4-9
<i>I. paradoxus</i> ♀ Nagoya n = 10	mean	28.85	32.65	29.40	52.60	29.00
	S.D.	2.03	2.00	1.92	3.41	2.37
	range	26-33	29-35	26-33	47-59	24-33

(continues)

(continued)

Species		No. of suckers on appendages				
		A1	A2	A3	TC	A4
<i>I. paradoxus</i> ♂ Nagoya n = 10	mean	25.80	29.30	27.25	47.85	7.15
	S.D.	3.50	3.14	3.51	6.76	1.20
	range	21-33	24-34	22-36	39-63	5-10
	Mean (♀)		29.98 ± 2.08		54.13 ± 3.39	
	Mean (♂)		27.08 ± 3.04		47.98 ± 5.05	
<i>I. pygmaeus</i> ♀ Mudong n = 10	mean	22.80	26.10	26.05	62.40	23.10
	S.D.	2.16	1.87	2.12	3.74	1.97
	range	19-28	21-28	22-30	54-71	20-27
<i>I. pygmaeus</i> ♂ Mudong n = 10	mean	18.65	22.20	21.95	53.35	2.65
	S.D.	1.61	1.99	2.00	2.48	0.80
	range	16-21	19-26	19-25	50-60	1-4
<i>I. pygmaeus</i> ♂ Indonesia n = 10	mean	17.30	20.70	19.75	44.40	2.65
	S.D.	1.59	1.69	1.50	2.37	0.50
	range	12-19	16-23	17-22	41-51	2-3
	Mean (♀)		24.98 ± 2.05		62.40 ± 3.74	
	Mean (♂)		20.09 ± 1.73		48.88 ± 2.43	
<i>I. thailandicus</i> ♀ n = 13*	mean	-	-	-	-	-
	S.D.	-	-	-	-	-
	range	20-24	20-26	18-26	34-45	19-28
<i>I. thailandicus</i> ♂ n = 11*	mean	-	-	-	-	-
	S.D.	-	-	-	-	-
	range	15-16	15-20	15-20	25-35	3-4

other investigated species of *Idiosepius*. Bites on arm pair I and II, on tentacles, and skin injuries on the appendages are frequent in this species; on average, every third animal is affected. Specimens with such injuries on the extremities were excluded from the evaluation.

Individuals of *Idiosepius paradoxus* have 54.13 ± 3.39 (♀) and 47.98 ± 5.05 (♂) suckers; while females from Ushimado have more suckers than those from Nagoya, for males the ranking is the opposite.

Idiosepius pygmaeus has 62.40 ± 3.74 (♀) and 51.84 ± 3.05 (♂) suckers, whereas male specimens from Indonesia have fewer suckers than individuals from Thailand.

Male *Idiosepius thailandicus* have 25–35 suckers on the tentacle club, females 34–45 suckers (Chotiyaputta et al., 1991).

Examining the number of suckers on the tentacle club by species indicates a high significance that may allow a differentiation of the two- and four-row group (Table 5). The significance within the four-row group is small but

provides no species-specific differentiation.

The female paratype of *I. macrocheir* with its 40 and 42 suckers resembles in its number more closely the two-row than the four-row group.

Hectocotylus Shape

In the genus *Idiosepius* both ventral arms in males are hectocotylized. The following schematic drawings (Fig. 4) provide an overview of the right and left ventral arms according to the literature references. The subsequent SEM images (Fig. 5A–J) give a more detailed picture of its outer shape.

In *Idiosepius biserialis*, both ventral arms are slender, the right ventral arm has two small flaps, separated by a deep cleft, at the tip (Fig. 5A), whereas the left one has a furrow on the aboral side (Fig. 5B). In some males, the hectocotyli arms were somewhat unequal in length; the left arm was shortly shorter than the right one.

Voss (1962) collected and described only a female individual of *Idiosepius macrocheir*; no

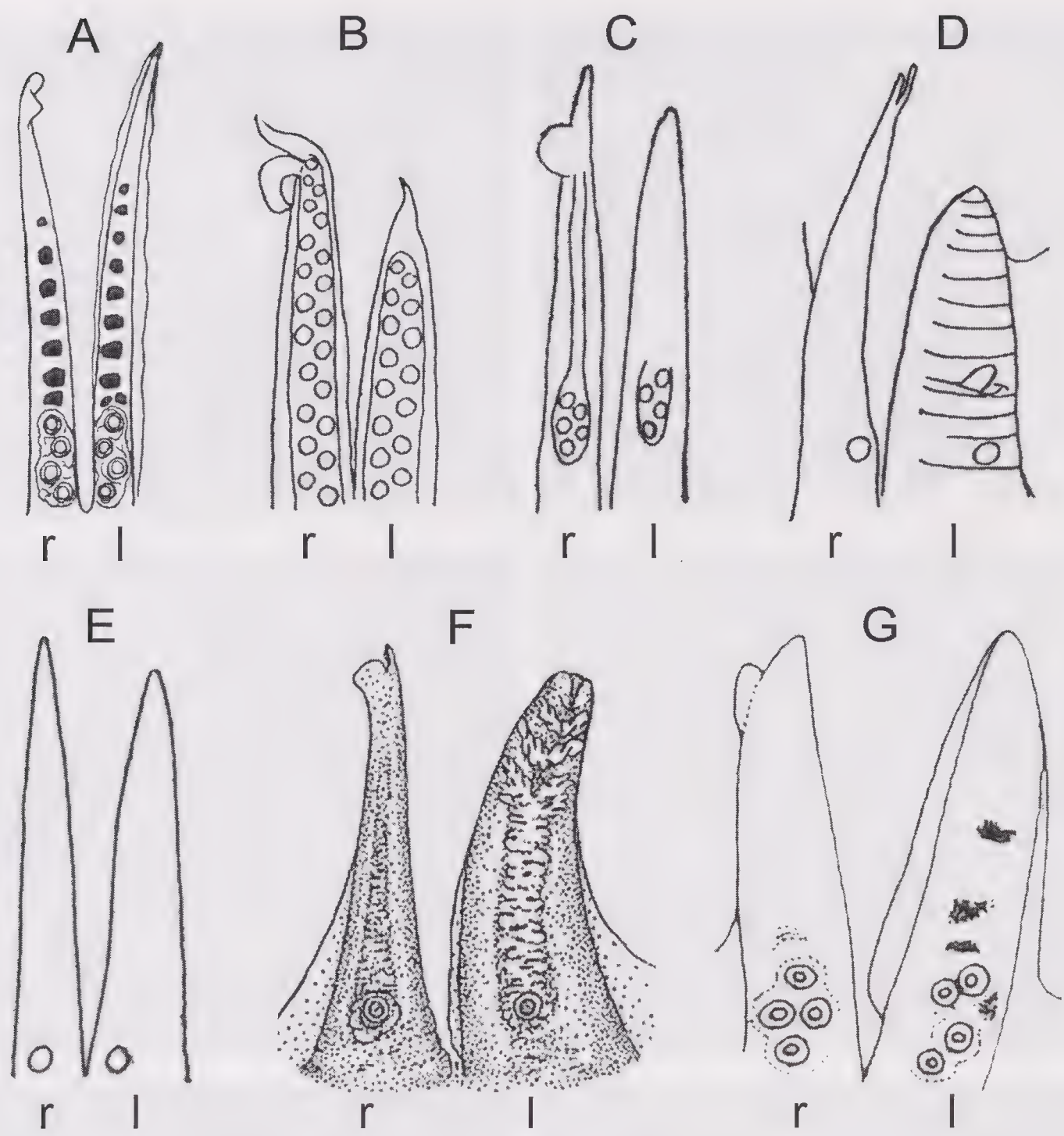


FIG. 4. Schematic drawings of the right (r) and left (l) hectocotylus from the original literature references. (A): Image of *I. biserialis* from Voss (1962); (B): Image of *I. notoides* from Berry (1921a); (C): Image of *I. paradoxus* from Sasaki (1914); (D): Image of *I. picteti* from Joubin (1894); (E): Image of *I. pygmaeus* from Steenstrup (1881); (F): Image of *I. pygmaeus* from Voss (1963); (G): Image of *I. thailandicus* from Chotiyaputta et al. (1991).

TABLE 5. Statistical differences in number of suckers in *Idiosepius* spp.

Species A	Species B	Mean Difference (A–B)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
<i>I. biserialis</i>	<i>I. notoides</i>	-27.417*	29.751	0.000	-36.168	-18.665
	<i>I. pygmaeus</i>	-16.958*	13.046	0.000	-20.632	-13.284
	<i>I. paradoxus</i>	-17.858*	15.411	0.000	-22.239	-13.478
<i>I. notoides</i>	<i>I. biserialis</i>	27.417*	29.751	0.000	18.665	36.168
	<i>I. pygmaeus</i>	10.458*	31.245	0.017	1.438	19.478
	<i>I. paradoxus</i>	9.558*	32.304	0.040	0.323	18.794
<i>I. pygmaeus</i>	<i>I. biserialis</i>	16.958*	13.046	0.000	13.284	20.632
	<i>I. notoides</i>	-10.458*	31.245	0.017	-19.478	-1.438
	<i>I. paradoxus</i>	-0.900	18.127	0.997	-5.942	4.142
<i>I. paradoxus</i>	<i>I. biserialis</i>	17.858*	15.411	0.000	13.478	22.239
	<i>I. notoides</i>	-9.558*	32.304	0.040	-18.794	-0.323
	<i>I. pygmaeus</i>	0.900	18.127	0.997	-4.142	5.942

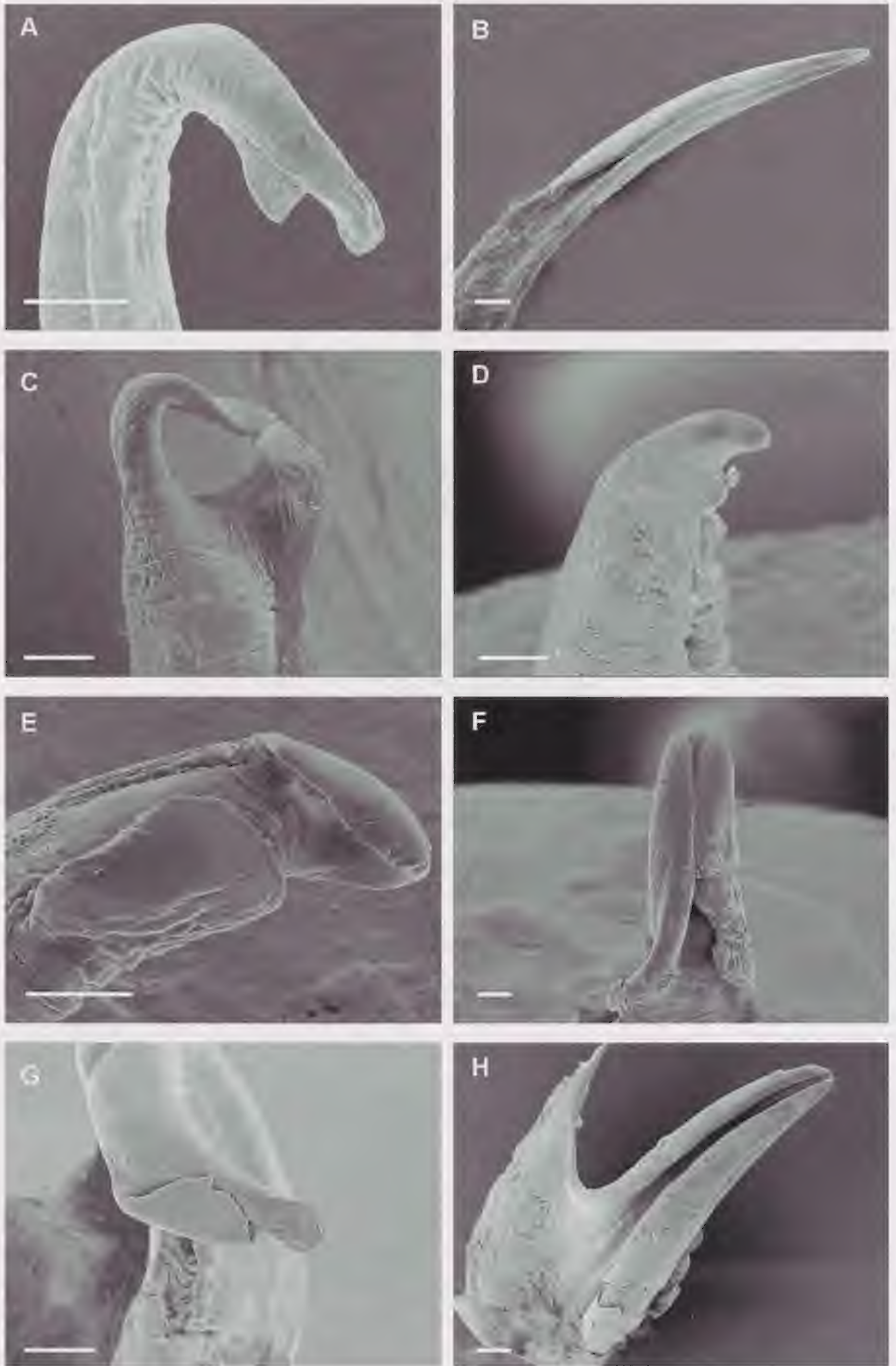


FIG. 5. SEM images of the hectocotyli of male *Idiosepius* spp. (A): Right ventral arm of *I. biserialis*; (B): Left ventral arm of *I. biserialis*; (C): Right ventral arm of *I. notoides*; (D): Left ventral arm of *I. notoides*; (E): Right ventral arm of *I. paradoxus*; (F): Left ventral arm of *I. paradoxus*; (G): Right ventral arm of *I. pygmaeus*; (H): Left ventral arm of *I. pygmaeus*. Scale bar = 200 μ m.

TABLE 6. Inter- and intraspecific variation in sucker number on both ventral arms in *Idiosepius* spp. (#Data by Joubin, 1894; *Data by Chotiyaputta et al., 1991).

Left/right ventral arm	<i>I. biserialis</i>				<i>I. notoides</i>	<i>I. paradoxus.</i>	<i>I. picteti</i> #	<i>I. pygmaeus</i>	<i>I. thailandicus*</i>	
	Moç.	Jap.	Ind.	Thail.				Thail.	Ind.	
	N = 52	N = 7	N = 1	N = 7	N = 20	N = 116	N = 1	N = 64	N = 40	N = 11
0/0								1		
1/1							1	2	1	
1/3	2							1		
1/4	1									
2/1	1									
2/2				1				5	6	
2/3	2							21	11	
2/4	1					1		1		
2/5	3									
3/2									4	
3/3	4							24	13	
3/4	6	1		2				7	4	11
3/5	12	4		1						
3/6	1									
3/7	1									
4/3	2							1		
4/4			1					1	1	
4/5	11	1		3		3				
4/6	1	1				3				
4/7						2				
4/8	1					1				
5/5	2					3				
5/6						12				
5/7						13				
5/8	1					7				
6/5						1				
6/6						7				
6/7						12				
6/8						16				
6/9						2				
6/10						2				
7/6						2				
7/7						5				
7/8						8				
7/9						8				
7/10						2				
8/9						2				
8/10						1				

(continues)

(continued)

	<i>I. biserialis</i>				<i>I. notoides</i>	<i>I. paradoxus.</i>	<i>I. picteti</i> #	<i>I. pygmaeus</i>	<i>I. thailandicus*</i>
Left/right	Moç.	Jap.	Ind.	Thail.				Thail.	Ind.
ventral arm	N = 52	N = 7	N = 1	N = 7	N = 20	N = 116	N = 1	N = 64	N = 40
									N = 11
8/11						1			
9/9						1			
9/12						1			
11/15					1				
11/19					1				
12/16					1				
12/17					1				
13/18					1				
13/19					2				
13/22					3				
14/19					1				
14/21					1				
15/19					1				
15/21					1				
15/23					2				
15/26					1				
16/25					1				
17/27					1				
18/20					1				

information about the modified ventral arms in males is available.

The right ventral arm of *Idiosepius notoides* is slightly longer than the left, with a few more suckers and with a double flap on its tip (Fig. 5C). The left ventral arm is small and broad, its tips singular end narrow and pointed without any appendages (Fig. 5D).

In *Idiosepius paradoxus* both ventral arms have the same length. The right one has a semicircular membrane on the dorsal side near the tip. The tip itself ends as a broad “cap-like” cover (Fig. 5E, F). The left ventral arm has fleshy ridges on the oral side and, as *I. biserialis*, a furrow on the aboral side (Fig. 5G), which was not mentioned in the original description and drawing.

According to Joubin (1894), the right ventral arm of *Idiosepius picteti* is slender, long and bilobated at the tip. He also reported that the left ventral arm in male *I. picteti* is very short and broad (Fig. 4D).

The description and morphology of the hectocotylus of *Idiosepius pygmaeus* corre-

sponds more to the schematic drawing of Voss (1963) (Fig. 4F) than to those of Steenstrup (1881) (Fig. 4E). As in *I. paradoxus*, also in *I. pygmaeus* both ventral arms have the same length. The right arm in *I. pygmaeus* is thinner and slender but two-lobed at its tip (Fig. 5H). The left ventral arm is stout and thick and has fleshy ridges on the oral and a furrow on the aboral side (Fig. 5I).

According to Chotiyaputta et al. (1991) (Fig. 4G), the right arm of *Idiosepius thailandicus* is much thicker and has a small membrane at its tip, while the left hectocotylized arm is slender with a distinct lateral membrane.

In some species – *I. biserialis* *I. paradoxus*, *I. pygmaeus*, *I. thailandicus* – the left ventral arm is furrow-like aborally, a feature absent in *I. notoides* and *I. picteti*. The right arm mostly carries appendages, such as membranes, or is modified at its tip. Although this characterization mostly corresponds to the description in the original literature, some aspects (description of *I. paradoxus*) are added. In summary, the shape of the fourth arm pair as well as

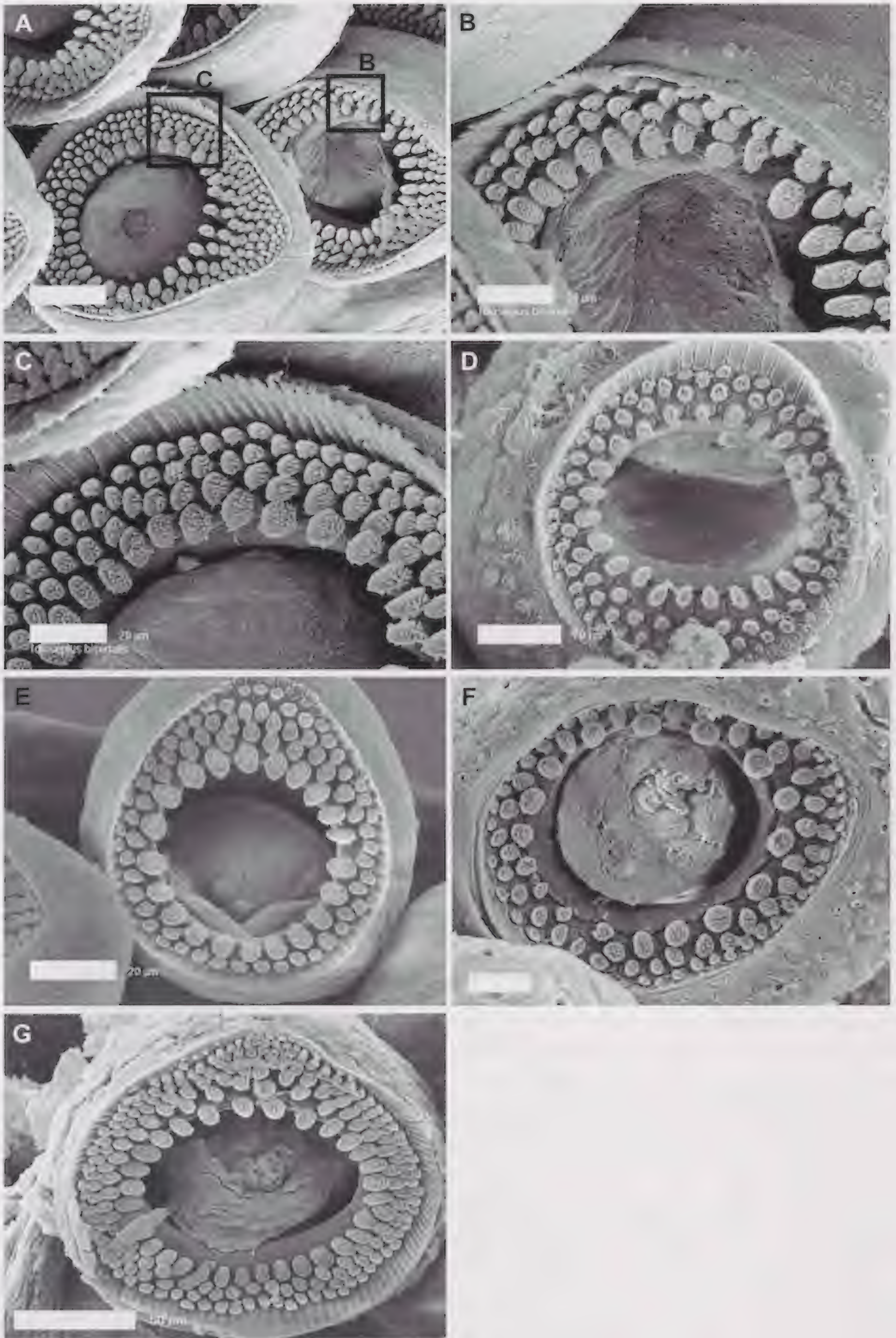


FIG. 6. Suckers on tentacle of *Idiosepius* spp. (A–C): *I. biserialis* (Mozambique) with a 3- (B) and 4-series (C) arrangement of pegs; (D, E): In some cases a 2- to 3-series arrangement of pegs on arm (D) or tentacle sucker (E) is present; (F, G): Suckers on arm (F) and tentacle (G) of *I. thailandicus* paratype show a 3-series arrangement of pegs. Scale bar = 50 μm (A, G); scale bar = 20 μm (B–F).

its appendices varies between the species and therefore provides a strong feature for its characterization.

Suckers on Hectocotylus

All investigated specimens are believed to have fully developed hectocotyli, except for certain individuals of *I. notoides* with partly malformed ventral arms. Strong inter- and intraspecific variation in sucker number was found on both ventral arms (Table 6): *Idiosepius biserialis* from Mozambique have 1–5 suckers on the left and 3–8 suckers on the right ventral arm. Predominant are the combinations 3/4, 3/5 and 4/5 suckers on the left/right ventral arms, but variations from 1/3 to 4/8, 5/5 and 5/8 are present. In the specimens from Thailand, 3/4 and 4/5 suckers on left/right ventral arms are also common. The individual from Indonesia has four suckers on each arm, whereas specimens from Japan mainly have three suckers on the left and five on the right ventral arm.

Idiosepius notoides has the highest number of suckers on the hectocotylized arms (11/15 to 18/20 suckers), whereas the combinations 13/19, 13/22, and 15/23 suckers are somewhat more common.

Idiosepius paradoxus exhibit the greatest variability in sucker combinations: 4–9 suckers on the left and 5–12 suckers on the right ventral arm. The combinations 5/6, 5/7, 6/7 or 6/8 suckers are most frequent. One individual has 2/4 suckers.

The male holotype of *Idiosepius picteti* has only one sucker on each ventral arm (Joubin, 1894).

Idiosepius pygmaeus (Mudong) sucker combinations vary from 0–4 suckers on the hectocotylized arms. More than 30% of the specimen have 2/3 suckers on the left/right or three suckers on both ventral arms. Specimens from Indonesia predominantly have two or three suckers on the left and three suckers on the right ventral arm; one individual has 1/1, another 4/4 suckers.

According to Chotiyaputta et al. (1991), male *Idiosepius thailandicus* have 3–4 suckers on the basal area of the hectocotylus. Variations in this species have not yet been described.

Within the two-row group, similar variations of sucker number are present and enable no clear distinction between *I. biserialis* and *I. thailandicus*. Due to the limited overlap in ranges of sucker numbers, the species (*I. notoides*, *I. paradoxus* and *I. pygmaeus*) of

the four-row group are well defined. A single sucker on the hectocotyli is present in *I. picteti*, *I. pygmaeus* from Thailand and *I. pygmaeus* from Indonesia.

Sucker Ultrastructure

All species of *Idiosepius* including the re-examined paratype of *I. thailandicus* (Fig. 6F, G) have suckers with mostly three or four rows of pegs in the suckers of the tentacles and arms. Two rows are also present in the suckers, although this is not only restricted to *I. thailandicus* but present in all *Idiosepius* species. Sometimes this variation arises on the same appendage (Fig. 6A–C) or even on a single sucker (Fig. 6D, E).

Not only in the paratype of *I. macrocheir*, as proposed by Voss, 1962, but also in *I. biserialis* and *I. thailandicus* the suckers in the middle

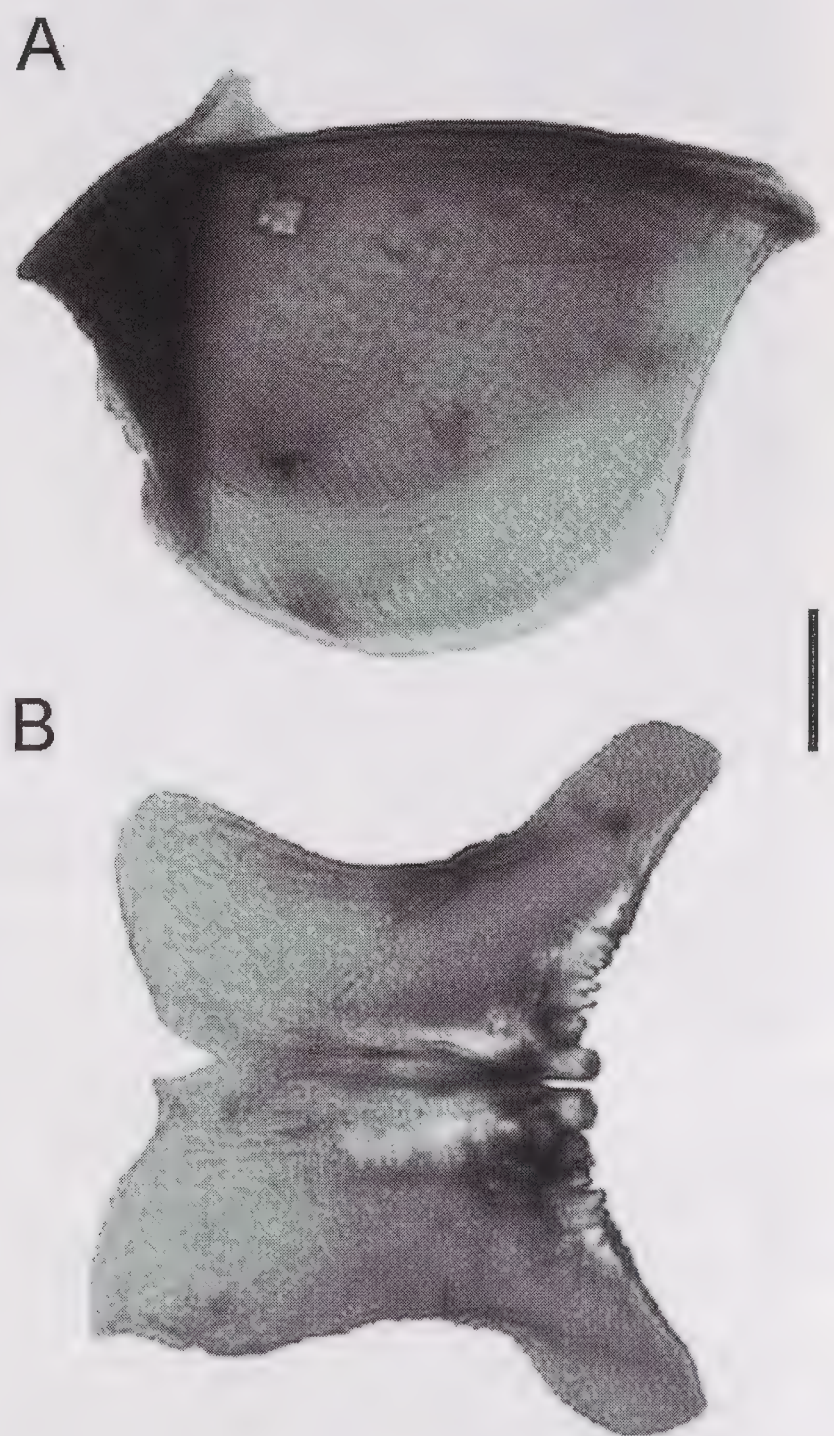


FIG. 7. Beaks of *Idiosepius* spp. (A): Side view of the upper beak from male specimen of *I. pygmaeus* (Bangrong); (B): Top view of the lower beak of male specimen of *I. paradoxus* (Nagoya). Scale bar = 252 μ m (A); scale bar = 248 μ m (B).

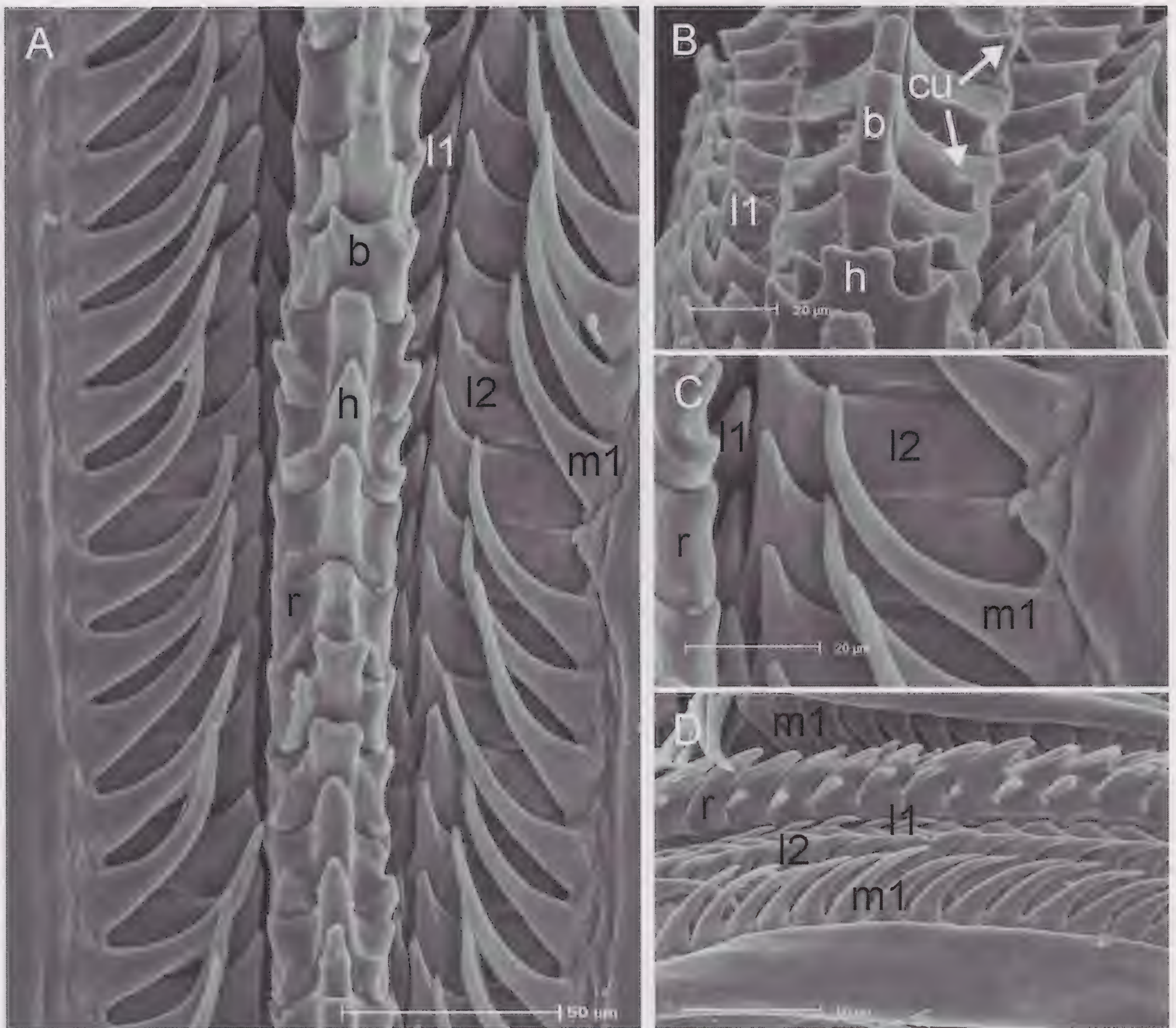


FIG. 8. Radula of *Idiosepius* spp. (A–C): Rhachidian teeth ((r) tall and homodont (h), compact and bidentate (b), cones and cusps (cu), two lateral teeth (l1 and l2) and the marginal tooth (m1)); (D): Radula seen from the right.

part of tentacle club are larger (about 1/4) than those at its base and tip, while in arms only the suckers at the tip are smaller. This attribute is lacking in the four-row species *I. notoides*, *I. paradoxus* and *I. pygmaeus*.

In all species, around every 20th individual of both sexes has only half-sized suckers in the middle part of the tentacle club (Fig. 10B). Since sucker pedicels were also frequently found during sucker evaluation, these are probably regenerated suckers.

Peg arrangement or sucker structure does not differ between the sexes and/or species. Our investigations show that the sucker form (and therefore its peg arrangement) is strongly influenced by fixatives, abnormal growth, and contraction of the arms and tentacles.

Beaks

The beaks in all species have a serrated cutting edge, apparent in both upper and lower beak (Fig. 7). The upper and lower beak has a black horny hood, whereas the lateral walls are colorless. Additionally, the upper mandible has a smooth inner rostrum, a short hood, which is low on the crest, and widely spread lateral walls, which have a shallow indentation of the posterior wall margin.

Although the two-row species *I. biserialis* from Mozambique and Thailand (*I. biserialis* from Japan and Indonesia were not evaluated) and *I. thailandicus* have smaller lower beaks than the four-row species, the evaluation provides no discernable differences of the upper beaks of any *Idiosepius* species.

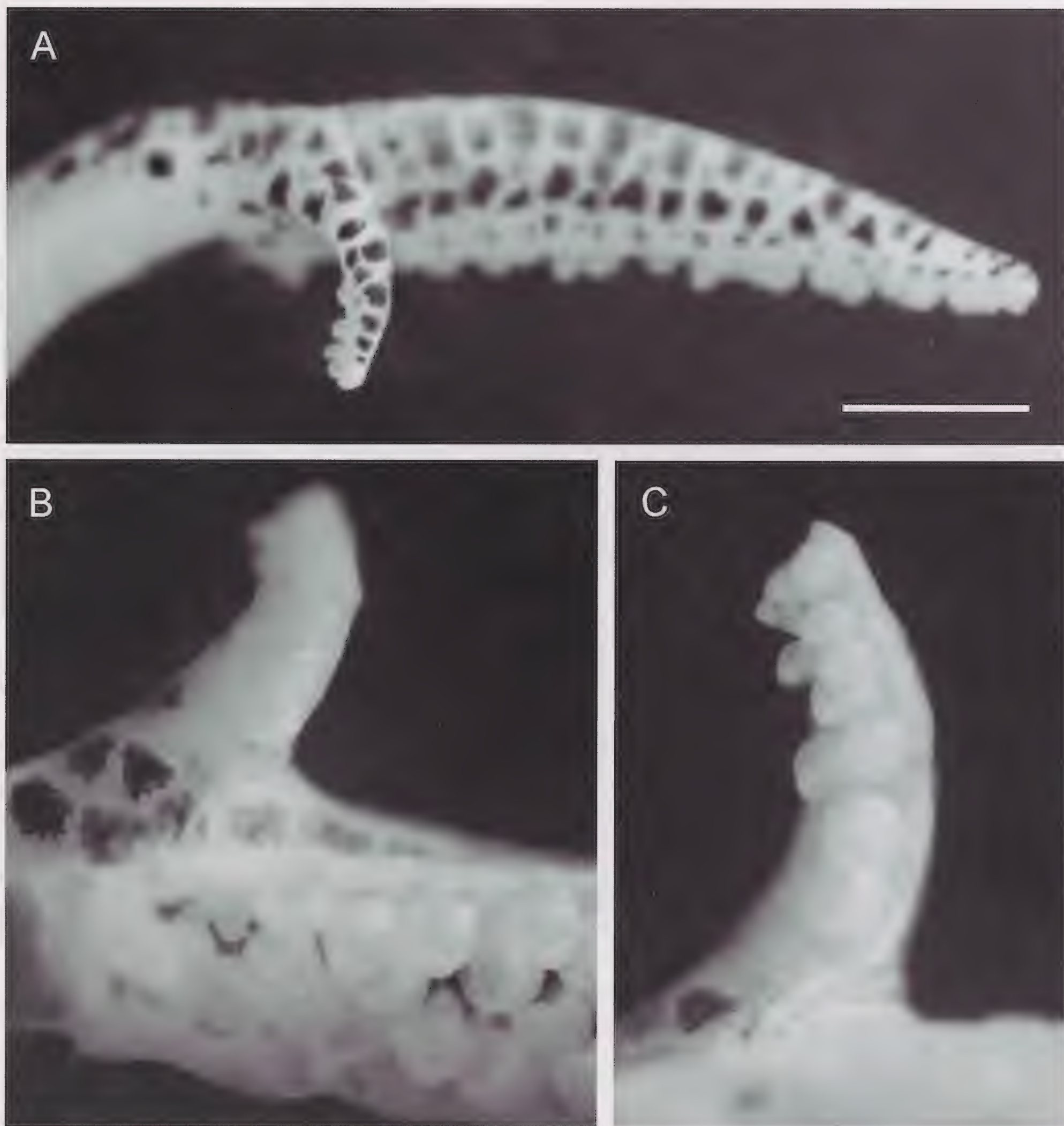


FIG. 9. Right tentacle of female *Idiosepius paradoxus*. (A): Overview; (B): Extra 'arm-like' structure; (C): Suckers. Scale bar = 1 mm.

Radula

The nomenclature of Nixon (1985) has been used in the following discussion. In all examined *Idiosepius* species ($n = 5$ of each species and location, except *I. biserialis* from Japan and Indonesia; *I. macrocheir* and *I. picteti* were not investigated), seven elements are present in the radula: the rhachidian tooth (*r*) is large. On either side of the rhachidian tooth, a first and

second lateral tooth (*l1* and *l2*) are present. Next to these, the marginal teeth (*m1*) complete the seven elements (Fig. 8A–D). Other elements such as a marginal plate are missing in this genus.

The rhachidian tooth exhibits a median cone with a series of tall homodont (*h*) or compact bidentate (*b*) cones. Cusps (*cu*) beside the cone are present in both types but they are more prominent in the bidentate type.

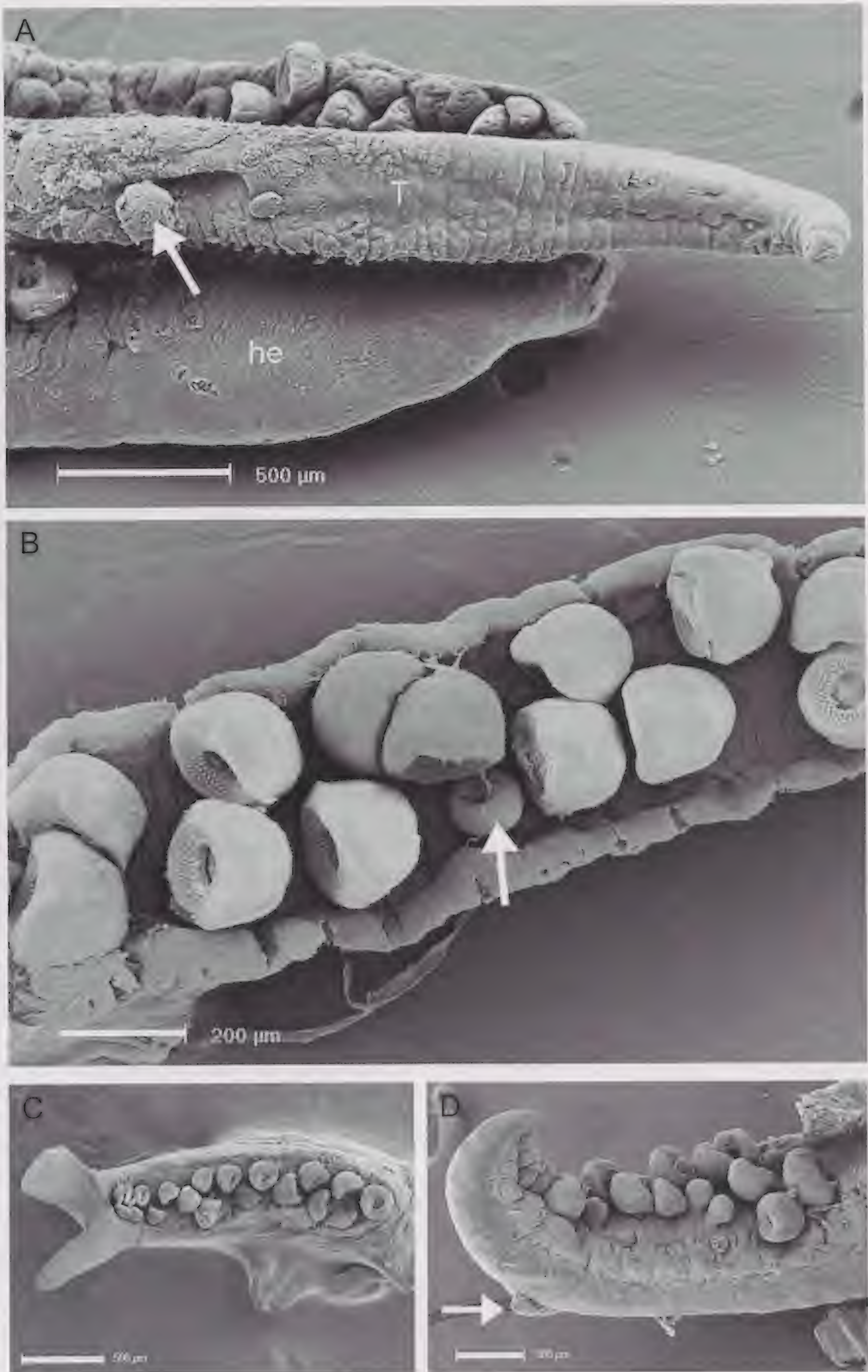


FIG. 10. Tentacles of *Idiosepius* spp. (A, B): Male *I. paradoxus* (A) with only one sucker (arrow) on the right tentacle (T) and adjacent hectocotylus (he). Residual structures like sucker shafts are absent (B). Tentacle with half-sized suckers are indicated by arrows; (C, D): In *I. notoides*, deformations in the right hectocotyli are visible. While the normal ventral arm has two small flaps (C), in some hectocotyli these appendages (arrow) are reduced or missing (D). Scale bar = 500 μm (A, C); scale bar = 200 μm (B, D).

The two lateral teeth are unicuspid but vary in shape and size. The tooth I1 is small, somewhat triangular and increases in height along of the ribbon. The lateral tooth I2 has longer cusps and is laterally ridged. The marginal tooth is sabre-shaped and the tallest element in the radula.

Within species, no differences were observed between the sexes in the arrangement or structure of the radula. Size differences of the teeth correlate with mantle length of the animals. Only the radula of *I. notoides* is distinct from the other species in the absence of bidentate cones on the rhachidian tooth.

Malformations

Apart from the fixation-induced body and/or tentacle deformation, abnormal growth was found in the tentacles and their suckers in *I. paradoxus* (Nürnberg et al., 2006). In one female, an extra “arm-like” structure branched basally from the right tentacle (Fig. 9A–C). This appendix was smaller than the tentacle, with suckers arranged irregularly or in pairs. One male developed only one sucker on the right and no suckers on the left tentacle. Even residual structures such as sucker stalks or injuries were not visible (Fig. 10A).

Malformation of the hectocotylus was recorded only in *I. notoides* specimens. While the normal right ventral arm has two flags at its tip (Fig. 10C), in many individuals (about 11 of 20 investigated specimens) the flaps were less strongly developed or missing (Fig. 10D).

DISCUSSION

Since the discovery of *I. thailandicus* by Chotiyaputta et al. (1991), questions have been raised about the taxonomic characters proposed by Nesis (1982). The aim of this study is to re-evaluate several morphological attributes in all nominal *Idiosepius* species and find reliable diagnostic characters that are valid for all.

The present study indicates that such highly plastic characters as mantle length, the counts and arrangement of suckers on the tentacle club, and other potential features (body shape, cornea fixability or contour of the adhesive organ) (Boletzky et al., 2005), fail to discriminate the species of *Idiosepius*. Handling and fixation strongly influence these characters, leading to misinterpretations for *Idiosepius* (Byern & Klepal, 2007) as well as for other cephalopod

groups (Allcock et al., 2008; O'Shea, 1997; Voight, 2001).

Even though the sucker arrangement on the extremities and its arrangement provides a reliable trait on taxa level for octopodids (Toll, 1988; Voight, 1993), a grouping of the *Idiosepius* species (two- and four-row), as done by Nesis (1982), seems to be less appropriate in view of induced sucker missarrangements and should therefore be rejected.

Also, the characterization of the *Idiosepius* species by sucker counts on the hectocotylus is of doubtful use. Earlier studies by Appellöf (1898), Sasaki (1914), and Hylleberg & Nateewathana (1991b), as well as the present study, pointed out that its number vary strongly leading to overlaps between some species (i.e., *I. biserialis* and *I. pygmaeus* or *I. picteti* and *I. pygmaeus*).

Of all here evaluated morphological attributes, only the shape of the ventral arms with its appendages (e.g., double flag, cup-like tip, etc.) allows a clear and reliable classification of the *Idiosepius* species. However, we are aware that this taxonomic classification is only applicable for males; female individuals, as in particular given for *I. macrocheir*, still remain indistinguishable based on this attribute.

The homodont rhachidian tooth should be used as an additional character to distinguish at least the females of *I. notoides* from the remaining species; nonetheless, we are still unable to find other morphological characteristics that would allow a unisex species classification.

Aside, the present study indicate that the characterization of *I. thailandicus* and *I. macrocheir* by the morphological criteria proposed by Chotiyaputta et al. (1991) and Voss (1962) are of doubtful use.

Idiosepius thailandicus provide no systematic relevant differences to *I. biserialis* either in hectocotylus shape and appendages, sucker size dimorphism, and peg arrangement on the tentacle clubs as well as other evaluated morphological attributes.

Due to the simultaneous and independent discovery of *I. thailandicus* (Chotiyaputta et al., 1991) and *I. biserialis* (Hylleberg & Nateewathana, 1991a) in Thailand, we suggest that the authors may have described the same species; thus, *I. thailandicus* was wrongly characterized as single species however rather represents individuals of *I. biserialis*.

This hypothesis is supported by cross-mating experiments (Nabhitabhata et al., 2006; Nabhitabhata & Suwanmala, 2008), in which individuals of *I. biserialis* and *I. thailandicus* mate

with each other, producing fertilized eggs from which embryos hatched. However, since cultivation of *Idiosepius* hatchlings in aquaria is still limited (Byern et al., 2006), it was not possible yet to verify the fertility of these *I. biserialis*-*I. thailandicus* hatchlings.

It also remains questionable whether both taxa are able to come together in nature. Since our knowledge about the genus *Idiosepius* is still marginal, relatively little is known about the biology, life cycle or geographical distribution of the species. Observations (Suwanmala et al., 2006; Byern & Klepal, 2007) indicate that *I. biserialis* and *I. pygmaeus* partly occur in neighbored areas, whether this is also valid for *I. biserialis* and *I. thailandicus* or other species remains unclear up to now.

Based on the sucker arrangement, the female holotype *I. macrocheir* belongs to the four-row group, as stated by Voss (1962). However, the sucker size dimorphism, sucker disarrangement as well as sucker number on the tentacle clubs place this species more towards the two-row species *I. biserialis*.

Based on our results, we suppose that *I. macrocheir* was likewise wrongly assigned as single species but rather represents an individual of *I. biserialis*. However, since the holotype of *I. macrocheir* is a female, a detailed characterization by the shape of the hectocotylized arms and their appendages, as given for the other *Idiosepius* species, is lacking in this case.

Additionally, several collection expeditions by the South African Museum in this region have been undertaken since Voss (1962). None yielded further individuals of *I. macrocheir* that would verify the presence of four-row individuals in African waters.

This study is a first attempt to compare all species of the genus *Idiosepius* and provide new insights into its relationships. Nevertheless, additional collections and evaluations as well as practical verification of the proposed systematic characterization based on the hectocotylus by other researchers are necessary to provide a more complete picture of the genus *Idiosepius* and, ultimately, to help re-organize its systematic key.

used in this research project: J. Nabhitabhata from Prince of Songkla University, Hat-yai and J. Suwanmala from Kasetsart University, Dept. of Marine Science, Bangkok, Thailand; M. Roeleveld from the South African Museum, Cape Town, South Africa; F. L. Ribeiro, J. Pais Murama, and R. de Silva from the Fisheries Research Institute, Dept. of Aquaculture, Maputo and P. Safrad, Manager of the Marine Biology Research Station on Inhaca Island, Mozambique; S. Shigeno from the University of Chicago, Dept. of Neurobiology, Pharmacology and Physiology, Chicago, USA, and T. Kasugai from the Port of Nagoya Public Aquarium, Nagoya, Japan; G. Pecl and J. Semmens from the Tasmanian Aquaculture and Fisheries Institute, University of Hobart, Australia, and M. Steers from the South Australian Research and Development Institute, Australia.

Our thanks go further to the staff from the South African Museum, Cape Town, Mr. Finet and his colleagues from the Muséum d'Histoire Naturelle, Genève, Mr. Laperousaz from the South Australian Museum, Sydney, and Dr. Kubodera from the National Science Museum, Tokyo, for providing specimens and holotype material for the current morphological and molecular biological investigations.

Special thanks go to the Austrian Science Fund (FWF, Project No. P 17 193 – B 12) and the Japan Society for the Promotion of Science (JSPS, Grant No. 04567), which enabled the collection of most species and funded the investigations. We thank Daniela Gruber and Gerhard Spitzer for their productive assistance during ultrastructural investigations. Mag. Sylvia Nürnberger gave us helpful comments and constructive criticism during the research project. The editorial assistance of the two anonymous reviewers, Dr. Michael Stachowitsch from the University of Vienna, Austria, Dr. Irene Zweimüller from the University of Vienna Austria, Dr. Jørgen Hylleberg from the University of Århus Denmark, especially Dr. Michael Vecchione from the National Museum of Natural History Washington and Dr. Janet Voight from the Field Museum, Chicago USA, for critically reading the manuscript are greatly appreciated.

ACKNOWLEDGMENTS

We would like to mention in particular our partners for their support and cooperation during our stays. Their experience and knowledge allowed the official collection of the animals

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Revised ms. accepted 29 June 2009